

Idiopyrgus Pilsbry, 1911 (Gastropoda, Tomichiidae): a relict genus radiating into subterranean environments

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<https://zoobank.org/DC99B66D-5862-44E4-B699-0A9CEFE8738F>

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Academic editor: Frank Köhler ♦ Received 5 September 2024 ♦ Accepted 17 October 2024 ♦ Published 8 November 2024

Abstract

The freshwater gastropod fauna in Brazil presently consists of circa 180 known species, though this is deemed an underestimate. The little-studied cavern faunas have been considered a potential source of undescribed species, particularly regarding the Truncatelloidea. Here, based on new collection efforts in caves in Bahia state, Brazil, we describe two new troglobitic species of *Idiopyrgus* (Tomichiidae): *Idiopyrgus eowynae* **sp. nov.** and *Idiopyrgus meriadoci* **sp. nov.** We improve upon the previous molecular phylogeny of this genus and take the opportunity to reclassify *Spiripockia umbraticola* Simone & Salvador, 2021, as *Idiopyrgus umbraticola* (Simone & Salvador, 2021), **comb. nov.** The genus *Idiopyrgus* now contains ten species, reinforcing the presence of Tomichiidae in Brazil as a relict lineage that largely radiated into cave environments. Furthermore, considering that so far only snapshots of their biology are known, we have collated all the information currently available from the literature and field observations on the cavernicolous species of *Idiopyrgus*. Notably, due to human action in the area where the two new species have been found, they can already be considered vulnerable.

Key Words

Brazil, Caenogastropoda, Gondwana, molecular phylogenetics, *Spiripockia*, troglotauna, Truncatelloidea

Introduction

The freshwater gastropod fauna in Brazil presently consists of circa 180 known species. However, this number is probably an underestimate due to the dearth of taxonomic studies focusing on this fauna when compared to their marine and terrestrial counterparts (Machado et al. 2023). Most studies on freshwater gastropods in Brazil focus, somewhat understandably, on invasive species and public health issues, the latter representing the vast majority of published papers (Simone 2006; Machado et al. 2023).

Among Brazilian freshwater snails, the poorly known cave faunas have been considered as a potential source of undescribed species (Salvador et al. 2022b). That is expected to be particularly important for members of the superfamily Truncatelloidea (Salvador et al. 2022b; Machado et

al. 2023). Some truncatelloidean cave species displaying striking shell morphology have recently been discovered (e.g., Simone 2012; Simone and Salvador 2021).

In a recent phylogenetic study, Salvador et al. (2022a) recognised the family Tomichiidae as a clade comprised of one relict genus from each of three continents: South America (genus *Idiopyrgus* Pilsbry, 1911, restricted to Brazil), Africa (*Tomichia* Benson, 1851), and Australia (*Coxiella* Smith, 1894). Salvador et al. (2022a) also reassessed the diversity of *Idiopyrgus* and described two new species in the genus from caves in eastern Brazil.

Here, based on new collections in caves in Bahia State, Brazil, we describe two additional species of *Idiopyrgus* and reassess the classification of one species of *Spiripockia* Simone, 2012. We also briefly review the current knowledge of the biology of cave-dwelling members of the genus.

Materials and methods

Collections were conducted in a small subterranean drainage at Gruna do Pedro Cassiano, a limestone cave located in the Serra do Ramalho karst area, Bahia state, north-eastern region of Brazil (Fig. 1). The drainage is over 1.5 km long but shallow, reaching just about 80 cm depth at most (pools in the distal part of the cave). The area where specimens occur has many submerged roots and lentic waters, formed by silt, with depths of 10–15 cm. Temperature and pH measured on-site varied from 21.3–22.4 °C and 7.5–8.2; the pH values are typical of karst drainages. The Serra do Ramalho karst area comprises plateaus within the Bambuí geomorphological group. Moreover, the limestone in the region forms outcrops that extend parallel to the Middle São Francisco River (Gallão and Bichuette 2018). The climate is tropical dry (=semi-arid) (“Aw”), with low annual precipitation of about 640 mm (Bedek et al. 2018). Vegetation consists of mesophytic and xeromorphic forests interspersed with “Cerrado” (savannah-like vegetation). Fig. 2 shows details of the cave environment, microhabitat, threats, and coexisting species of the two new *Idiopyrgus* spp. Common threats in the region are related to human occupation (Gallão and Bichuette 2018), including: water exploitation for agriculture, land use lacking sustainable planning, and reduction in subterranean water bodies likely due to climate change, representing an alarming concern (MEB, pers. obs). Future mining projects are also expected for the region, which should cause severe impacts.

The collections were conducted in November 2021, under permission from Sistema de Autorização e Informação em Biodiversidade (SISBIO #28992). The collected specimens were euthanised, fixed, and preserved in 95% ethanol and deposited in the following collections: Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos (LES; São Carlos, SP, Brazil), Museu de Zoologia da Universidade de São Paulo (MZSP; São Paulo, SP, Brazil), and Museu Nacional (MNRJ; Rio de Janeiro, RJ, Brazil). Registration numbers of the specimen lots are given below in the entry for each species.

Molecular data

Selected specimens were used for DNA extraction, which was conducted using the QIAGEN DNEasy® Blood & Tissue Kit generally following the manufacturer’s standard protocol, but with a modification to the final step to increase yield and concentration, which is helpful for such small freshwater snails. The modification of the final step consisted of initially using one-quarter of the suggested amount of the buffer solution (i.e., 50 µL of buffer AE) and then repeating the elution step using the extract.

A total of six specimens were chosen for DNA extraction. Five of them were from the collection efforts in Gruna Pedro Cassiano (see above), as follows: 2 specimens of the larger-sized *Idiopyrgus* sp. nov. (a typical specimen and a dark morph) (from lots LES 29796 and

LES 29797), 3 specimens of the smaller-sized *Idiopyrgus* sp. nov. (from lot LES 29820). The sixth specimen was an individual of *Idiopyrgus souleyetianus* Pilsbry, 1911, stemming from local survey efforts in Minas Gerais state and kindly provided to us by Paulo Ricardo Coelho from the Federal University of Minas Gerais (UFMG, Brazil), included in the present study to improve the phylogenetic analysis. The extraction process failed for the three of the smaller-sized *Idiopyrgus* sp. nov., which were consumed in the process. So, we refrained from spending any additional specimens of it for DNA extraction, considering it better to preserve the scarce specimens with soft parts available for the future, when better options might become available.

We selected three genetic markers to target based on those used in previous phylogenetic studies done on the group (e.g., Kameda and Kato 2011; Wilke et al. 2013; Salvador et al. 2022a; Lawrie et al. 2023): (1) the barcoding region of the mitochondrial COI gene (primers LCO and HCO of Folmer et al. 1994); (2) the mitochondrial 16S rRNA gene (primers 16SarL and 16SbrH of Simon et al. 1994); (3) a stretch of nuclear DNA containing the 3’ end of the 5.8S rRNA gene, the entire ITS2 region, and a large portion of the 5’ end of the 28S rRNA gene, sequenced in two parts (primers LSU-1 and LSU-3 for the first part and primers LSU-2 and LSU-5 for the second part; Wade and Mordan 2000; Wade et al. 2006).

The same PCR protocol was used for amplification of COI and 16S, consisting of an initial denaturation step of 3 min at 95 °C, 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 1 min, and extension at 72 °C for 2 min; a final extension step of 5 min at 72 °C. The PCR protocol for the ITS2+28S section was as follows: initial denaturation step of 3 min at 95 °C; 40 cycles of denaturation at 95 °C for 30 s; annealing at either 50 °C (ITS2 portion) or 45 °C (28S portion) for 1 min; and extension at 72 °C for either 5 min (ITS2 portion) or 2 min (28S portion); final extension step of 4 min at 72 °C.

Success of PCR was assessed visually via agarose gel electrophoresis. The PCR products were then cleaned with ExoSAP-IT™ (Affymetrix Inc.) following the manufacturer’s protocol, and the samples were sent to Macrogen Europe (Amsterdam, The Netherlands) for Sanger sequencing. The resulting sequences were quality-checked and assembled using the software Geneious Prime (v.2023.2.1, Biomatters Ltd.). Consensus sequences were extracted and uploaded to GenBank; the accession numbers can be seen in Table 1.

Phylogenetic analysis

Our initial morphological assessment of the specimens suggested a placement in the genus *Spiripockia*, as they seemed closely related to the species *Spiripockia umbraticola* Simone & Salvador, 2021, previously described from another cave in Bahia state in the same area. To test that hypothesis, we included the new species into a phylogenetic framework focusing on the families of freshwater truncatelloidean snails present in

Brazil (Tateidae and Tomichiidae), as well as those families in which Brazilian species have been previously or tentatively classified (Pomatiopsidae and Cochliopidae). To that end, we relied on genetic data available from our

previous studies and those of other authors. The taxa used in the phylogenetic analysis are listed in Table 1, including information on GenBank accession numbers and references.

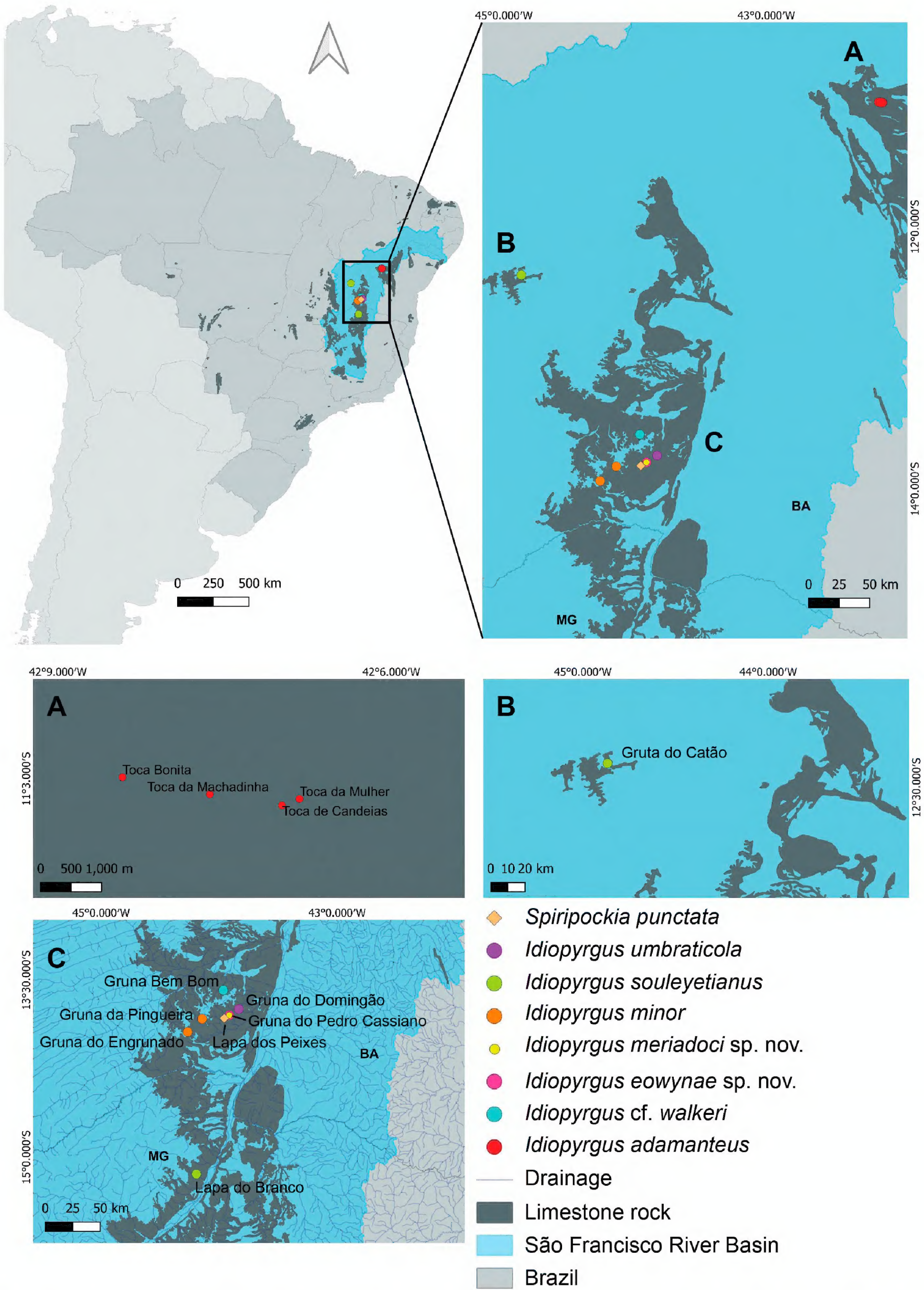


Figure 1. Distribution map of *Idiopyrgus* spp. (and *Spiripockia punctata*) in caves of the state of Minas Gerais and Bahia, Brazil.

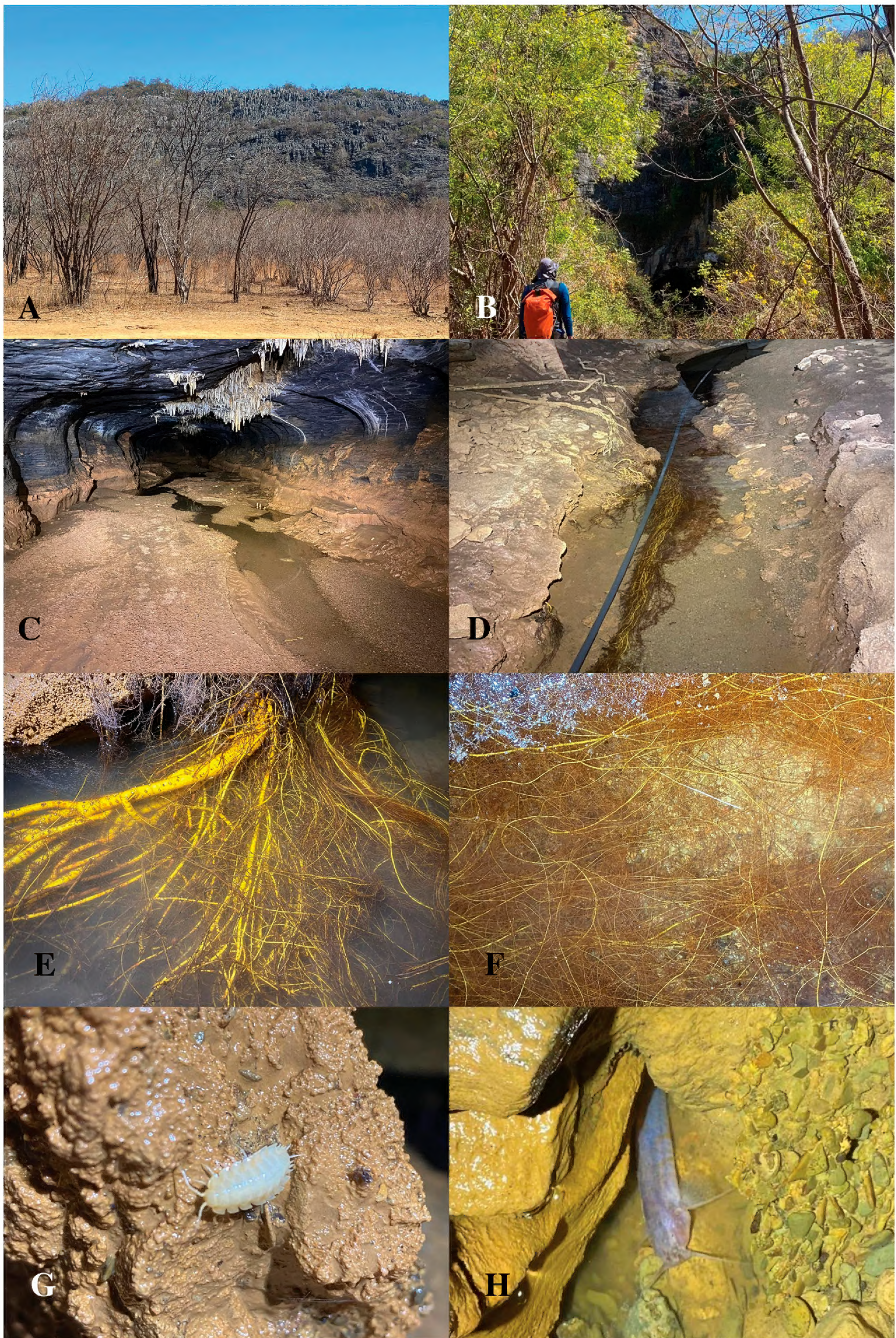


Figure 2. Gruna do Pedro Cassiano (“Pedro Cassiano cave”), Serra do Ramalho karst area, Carinhanha municipality, Bahia state, Brazil; **A.** Cave surroundings showing the limestone outcrops and the dried vegetation, sparse and altered by human impacts; **B.** Cave entrance.; **C.** Cave gallery showing the small subterranean drainage; **D.** Detail of the drainage with the apparatus of water extraction; **E.** Submerged roots, microhabitat of *Idiopyrgus* spp.; **F.** Detail of the microhabitat of *Idiopyrgus* spp., showing thinner roots; **G.** *Xangoniscus* aff. *aganju*, an amphibious troglotic isopod that co-exists with *Idiopyrgus* spp.; **H.** *Trichomycterus rubbioli*, a cave catfish that co-exists with *Idiopyrgus* spp.

Table 1. Species used in the phylogenetic analysis, with GenBank registration number of each genetic marker, locality data, and reference to the original publication. An asterisk after a species’ name indicates that it is the type species of its genus.

Taxon	COI	16S	28S	Locality	References
Amnicolidae					
<i>Akiyoshia kobayashii</i> Kuroda & Habe, 1958	AB611823	AB611822	AB611821	Japan, Shiga, Taga	Kameda and Kato 2011
<i>Amnicola limosus</i> (Say, 1817)	AF213348	AF212903	—	USA, Michigan, Blind Lake	Wilke et al. 2001
Assimineidae					
<i>Assimineia grayana</i> Fleming, 1828*	HQ623170	HQ623153	—	Germany, Lower Saxony, Varel	Wilke et al. 2013
<i>Paludinellassimineia japonica</i> (Pilsbry, 1901)*	AB611811	AB611810	AB611809	Japan, Ehime, Hiburī Is.	Kameda and Kato 2011
Cochliopidae					
<i>Eupaludestrina stagnorum</i> (Gmelin, 1791)	JQ973024	JX970535	—	The Netherlands, Zeeland, Zierikzee	Kroll et al. 2012; Wilke et al. 2013
<i>Helebops carrikeri</i> Davis & McKee, 1989	AF213347	AF212902	—	USA, Maryland, Dorchester	Wilke et al. 2000
<i>Mexipyrgus carranzae</i> Taylor, 1966*	AF129325	JX970534	—	Mexico, Coahuila, Mojarral Oeste	Hershler et al. 1999; Wilke et al. 2013
<i>Onobops jacksoni</i> (Bartsch, 1953)	AF367645	EU573990	—	USA, Maryland, Dorchester	Wilke et al. 2001; Ponder et al. 2008
<i>Spurwinkia salsa</i> (Pilsbry, 1905)*	AF367633	EU573991	—	USA, Maryland, Dorchester	Wilke et al. 2001; Ponder et al. 2008
Pomatiopsidae					
<i>Blanfordia japonica</i> (Adams, 1861)*	AB611727	AB611726	AB611725	Japan, Niigata, Sado	Kameda and Kato 2011
<i>Cecina manchurica</i> Adams, 1861*	AB611743	AB611742	AB611741	Japan, Ishikawa, Nanao	Kameda and Kato 2011
<i>Fukuia kurodai</i> Abbott & Hunter, 1949*	AB611767	AB611766	AB611765	Japan, Fukui, Takeda	Kameda and Kato 2011
<i>Gammaticula shini</i> (Habe, 1961)	AB611799	AB611798	AB611797	Japan, Okinawa, Yonaguni Is.	Kameda and Kato 2011
<i>Neotricula aperta</i> (Temcharoen, 1971)*	AF531541	AF531556	AY207034	Mekong River (28S Thailand, 16S/COI Laos)	Attwood et al. 2003
<i>Tricula bollingi</i> Davis, 1968	AF531553	AF531551	AY207039	Thailand, Chiang-Mai	Attwood et al. 2003
Tateidae					
<i>Potamolithus ribeirensis</i> Pilsbry, 1911	JX970618	JX970549	—	Brazil, São Paulo, Iporanga	Wilke et al. 2013
<i>Potamopyrgus antipodarum</i> (Gray, 1843)	AY631102	AY314009	AY014159	undetermined	Guan et al. 2008
<i>Potamopyrgus estuarinus</i> Winterbourn, 1970	AB930485	—	AB930357	New Zealand, Auckland, Orewa	Takano and Kano 2014
<i>Tatea huonensis</i> (Tenison Woods, 1876)*	JX970619	JX970550	—	Australia, New South Wales, Manly Lagoon	Wilke et al. 2013
Tomichiidae					
<i>Coxiella exposita</i> (Iredale, 1943)	ON426511	ON455985	ON456011	Australia, Western Australia, Cranbrook	Lawrie et al. 2023
<i>Coxiella gilesi</i> (Angas, 1877) sensu lato	ON426653	ON455993	ON456021	Australia, Western Australia, Lake Carnegie	Lawrie et al. 2023
<i>Coxiella glabra</i> Macpherson, 1957	ON426716	ON455989	ON456030	Australia, Western Australia, Three Springs	Lawrie et al. 2023
<i>Coxiella glauerti</i> Macpherson, 1957	ON426565	ON456001	ON456014	Australia, Western Australia, Esperance	Lawrie et al. 2023
<i>Coxiella</i> cf. <i>minima</i> Macpherson, 1954	ON426698	ON455991	ON456027	Australia, Western Australia, Lake Shaster	Lawrie et al. 2023
<i>Coxiella pyrrhostoma</i> (Cox, 1868)	ON426604	ON455998	ON456016	Australia, Western Australia, Esperance	Lawrie et al. 2023
<i>Coxiella striata</i> (Reeve, 1842)	ON426791	ON455990	ON456034	Australia, Victoria, Willaura	Lawrie et al. 2023
<i>Coxiella striatula</i> (Menke, 1843)*	ON426634	ON456004	ON456018	Australia, Western Australia, Esperance	Lawrie et al. 2023
<i>Idiopyrgus adamanteus</i> Salvador, Silva & Bichuette, 2022	ON323464	ON359913	ON324828	Brazil, Bahia, Central	Salvador et al. 2022
<i>Idiopyrgus minor</i> Salvador, Silva & Bichuette, 2022	ON720330	ON720564	ON720562	Brazil, Bahia, Feira da Mata	Salvador et al. 2022
<i>Idiopyrgus souleyetianus</i> Pilsbry, 1911*	—	PQ461199	PQ481827	Brazil, Minas Gerais, Baldim	this paper
<i>Spiripockia eowynae</i> sp. nov. (typical morph)	PQ462606	—	PQ481828	Brazil, Bahia, Carinhanha	this paper
<i>Spiripockia eowynae</i> sp. nov. (black morph)	PQ462605	—	PQ481826	Brazil, Bahia, Carinhanha	this paper
<i>Tomichia</i> sp.	ON426742	ON456007	ON456023	South Africa, Western Cape, c. Quoin Point	Lawrie et al. 2023
<i>Tomichia differens</i> Connolly, 1939	ON426746	ON456009	ON456024	South Africa, Western Cape, Bredasdorp	Lawrie et al. 2023
<i>Tomichia ventricosa</i> (Reeve, 1842)*	ON426774	ON456006	ON456026	South Africa, Western Cape, Soetendalsvlei	Lawrie et al. 2023
Truncatellidae					
<i>Truncatella pfeifferi</i> Martens, 1860	AB611819	AB611818	AB611817	Japan, Ishikawa, Nanao	Kameda and Kato 2011
<i>Truncatella subcylindrica</i> (Linnaeus, 1767)*	—	KC110035	KC109982	Italy, Sicily, Trapani	Criscione and Ponder 2013
Littorinidae (OUTGROUP)					
<i>Littoraria pallescens</i> (Philippi, 1846)	AB611831	AB611830	AB611829	Japan, Okinawa, Nago	Kameda and Kato 2011

Alignment of genetic sequences of each marker was conducted in Geneious Prime using the MAFFT plugin (Katoh et al. 2002; Katoh and Standley 2013) with default settings. The resulting alignments were visually proofed for inconsistencies and then run through Gblocks (Castresana 2000; Talavera and Castresana 2007) to eliminate poorly aligned or data-deficient positions that could affect the analysis. The resulting post-Gblocks alignments were concatenated (1779 bp in total: COI 638 bp, 16S 515 bp, ITS2+28S 626 bp) for a Bayesian inference phylogenetic analysis.

The analysis was conducted with MrBayes (v.3.2.7, Ronquist et al. 2012) through the CIPRES Science Gateway (Miller et al. 2015) with two concurrent runs, each with four Markov chains of 80 million generations (the first 20% discarded as ‘burn-in’), the default priors, nst = 6, rates = invgamma, temperature parameter = 0.1, sampling every 1,000 generations, and with substitution model parameters unlinked across the markers (COI, 16S, ITS+28S). MCMC convergence was assessed by examining the standard deviation of split frequencies (<0.001) and the potential scale reduction factor (PSRF ~1.0), as well as trace plots in Geneious (Ronquist et al. 2009).

Results

The resulting tree from the Bayesian analysis (Fig. 3) shows well-supported clades at the family level, as expected. It corroborates recent studies (Salvador et al. 2022a; Lawrie et al. 2023) that joined three relict genera from three different continents in the family Tomichiidae (Fig. 3: posterior probability PP = 1). Likewise, a sister taxon relationship was recovered between the African *Tomichia* and the Brazilian *Idiopyrgus*, albeit with low support (Fig. 3: PP = 0.72), as in the study of Lawrie et al. (2023). Within *Idiopyrgus*, *I. souleyetianus* is sister to a clade that includes all its sequenced congeners, all of which are cavern-dwelling species (Fig. 3: PP = 1 for the genus and all subclades). The latter includes the two specimens of the larger-sized *Idiopyrgus* sp. nov.: the typical morph and the dark morph. This new species and the related smaller-sized *Idiopyrgus* sp. nov. are described below as *Idiopyrgus eowynae* sp. nov. and *Idiopyrgus meriadoci* sp. nov. Unique and consistent sets of diagnostic conchological features are provided.

Pairwise sequence identity for the COI marker of *Idiopyrgus* spp. was extracted from the marker's alignment in Geneious Prime, ignoring eventual terminal gaps.

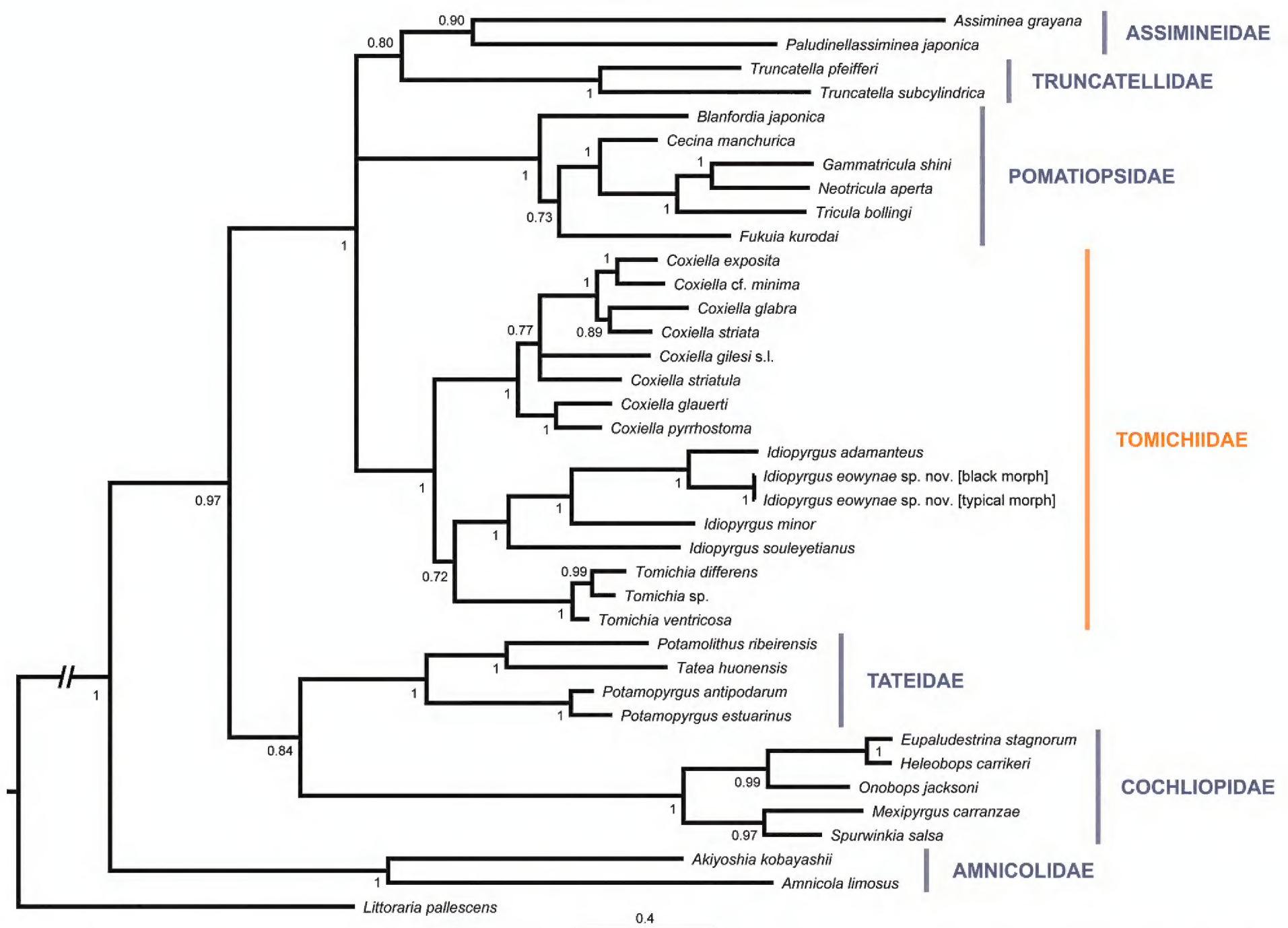


Figure 3. Bayesian inference phylogenetic tree based on concatenated but unlinked markers, highlighting the family Tomichiidae and showing the position of the new species in the genus *Idiopyrgus* and its relationship to its congeners. Posterior probabilities are shown on the nodes. Scale bar is substitutions per site.

The identity between the sequences of the typical and dark morphs of *I. eowynae* sp. nov. was 100%; between both morphs of *I. eowynae* sp. nov. and *I. adamanteus*, 90.8%; and between both morphs of *I. eowynae* sp. nov. and *I. minor*, 89.5%. No good-quality COI sequence could be obtained for *I. souleyetianus* despite repeated attempts.

Systematics

Family Tomichiidae

Genus *Idiopyrgus* Pilsbry, 1911

Idiopyrgus eowynae sp. nov.

<https://zoobank.org/733E29CA-FF4B-4138-A3A9-05396C501716>

Fig. 4

Type material. LES 29795 (holotype), LES 29796 (4 paratypes), LES 29797 (2 dark morphs paratype), MNRJ 37168 (3 paratypes), MZSP 168419 (3 paratypes). All specimens collected on 10.ix.2021 (M.E. Bichuette & J.E. Gallão leg.); all paratypes from type locality (except dark morphs, which are from the same cave but from the entrance zone).

Type locality. Brazil, Bahia state, Carinhanha municipality, Gruna do Pedro Cassiano (“Pedro Cassiano Cave”), 13°47'48.0"S, 43°54'50.0"W.

Etymology. The species is named in honour of Éowyn, from J.R.R. Tolkien’s “The Lord of the Rings.” Éowyn exemplifies courage, resilience, and resistance against darkness, both internal and external, standing against Gríma Wormtongue and the Witch-king of Angmar.

Diagnosis. Shell conical to turritiform and relatively tall in comparison to congeners. Body whorl is translucent yellow; previous whorls are slightly darker and brownish. Teleoconch sculpture consisting of few (4–7) radial rows of thorn-like hairs on the apical portion of whorl and a multitude of fine irregular spiral lines below them, reaching all the way to the umbilicus. Aperture large, rounded.

Description. Shell conical to turritiform, 5–5¼ whorls, 4.5–4.6 mm high, ~2.7 mm wide. Colour pale beige or yellowish on body whorl, but slightly darker and brownish on earlier whorls; body whorl translucent; early whorls typically with flaked off periostracum. Protoconch of 1¼ whorl, rounded, smooth (Fig. 4G, I). Suture deep. Whorls increasing uniformly in width but more rapidly in height. Teleoconch sculptured by minute triangular thorn-like ‘periostracal hairs’ arranged in equidistantly spaced spiral rows (4 to 7 rows) and restricted to apical portion of body whorl (Fig. 4A–D, H). Below the rows of periostracal hairs, there is a multitude of fine, irregular spiral lines, reaching all the way to the umbilicus (Fig. 4H). Periostracal hairs can be worn out in older specimens (Fig. 4C, D), particularly in the early whorls. Peristome complete, not covering penultimate whorl, expanded, wider in abapical region, narrower in columellar region, of same colour as body whorl or slightly whiter. Aperture rounded, with light apical angulation, but with smooth, rounded con-

tour. Umbilicus rimate, nearly closed. Operculum oval, paucispiral; corneous, thin, and translucent, of same colour as body whorl. Soft body completely white in colour.

Dark morph (Fig. 4E, F) differs from the above-described typical morph by having a black shell with thicker walls, in which the teleoconch sculpture is nearly invisible: a few remnants of the spiral rows of hairs are visible in the adapical portion of the whorls; traces of the sculpture can also be seen through the shell wall of the aperture (Fig. 4E).

Distribution. Known only from type locality (Fig. 1), the twilight and dark (aphotic) zones of the cave (typical morph) and at the cave entrance (dark morph).

Remarks. *Idiopyrgus eowynae* sp. nov. can be easily distinguished from most of its congeners (including the type species *I. souleyetianus*) by its much wider shell and the presence of teleoconch sculpture. It closely resembles *I. meriadoci* sp. nov. (see below) and *I. umbraticola*. It differs from *I. meriadoci* sp. nov. by a much larger size and larger number of whorls, as well as by having a different teleoconch sculpture, which includes periostracal hairs. Conchologically, *I. eowynae* sp. nov. is most similar to *I. umbraticola* comb. nov., from which it can be distinguished by its smaller size (~4.5 mm vs. ~5.0 mm in *I. umbraticola*), higher whorls with a more strongly convex outline, a larger and rounder aperture, and by having a different teleoconch sculpture. In *I. eowynae* sp. nov., there are few spiral rows of the triangular thorn-like hairs, restricted to the apical portion of the whorl, and which are then replaced by irregular spiral lines that continue towards the umbilicus (also visible in juveniles). In *I. umbraticola*, there is a larger number of rows of hairs, stretching the median portion of the whorl; the spiral lines are absent. Notably, a dark morph has also been observed in *I. adamanteus* Salvador, Silva & Bichuette, 2022, and *I. cf. walkeri* Pilsbry, 1924 (Salvador et al. 2022a).

Except for the existence of two morphs (typical and black), there is little variation in shell shape, form, and proportions among the presently available specimens of *I. eowynae* sp. nov. (Fig. 4A–F). Notably, the sequences of the COI barcoding gene fragment of the two morphs were 100% identical, while the sequences of both morphs had a pairwise identity of around 90% in relation to *I. adamanteus* and *I. minor*. According to the results of the phylogenetic analysis (Fig. 3), *I. eowynae* sp. nov. is sister to *I. adamanteus*; both species together are sister to *I. minor*.

Idiopyrgus meriadoci sp. nov.

Fig. 5

<https://zoobank.org/B70A02D0-9ABD-42F4-89FD-DE52950C7E44>

Type material. LES 29798 (holotype), LES 29820 (4 paratypes), MZSP 168420 (2 paratypes). All specimens collected on 10.ix.2021 (M.E. Bichuette & J.E. Gallão leg.); all paratypes from type locality.

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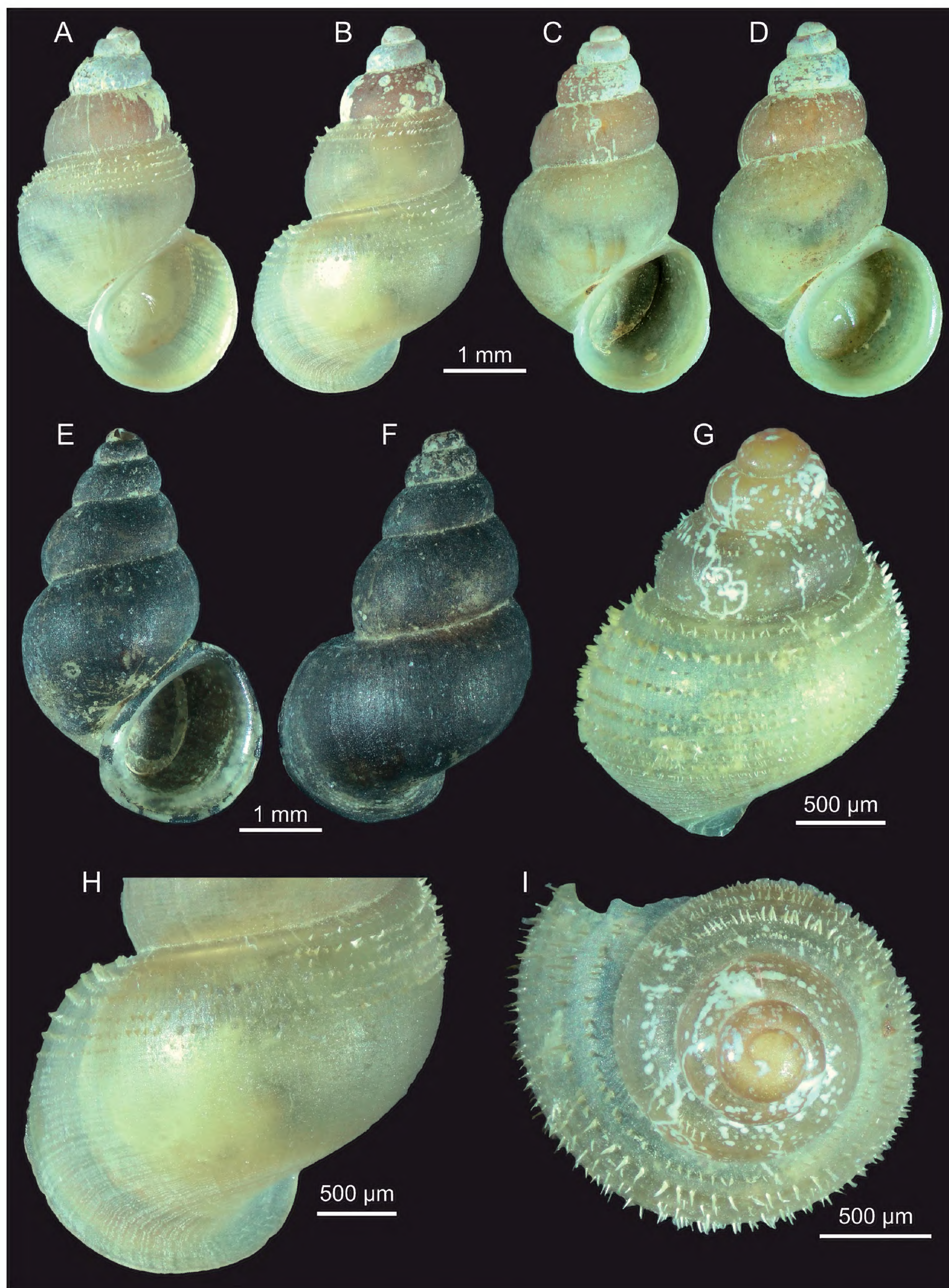


Figure 4. *Idiopyrgus eowynae* sp. nov. **A, B.** Holotype LES 29795; **C.** Paratype (typical morph) #1 LES 29796; **D.** Paratype (typical morph) #2 LES 29796; **E, F.** Paratype (dark morph) LES 29797; **G.** Paratype (typical morph) #3 LES 29796, juvenile, showing the protoconch in greater detail as well as the sculpture of the first teleoconch whorls; **H.** Detail of the body whorl of holotype in dorsal view, showing the teleoconch sculpture, in particular the multitude of spiral lines; **I.** Paratype #3 in apical view, showing the protoconch.

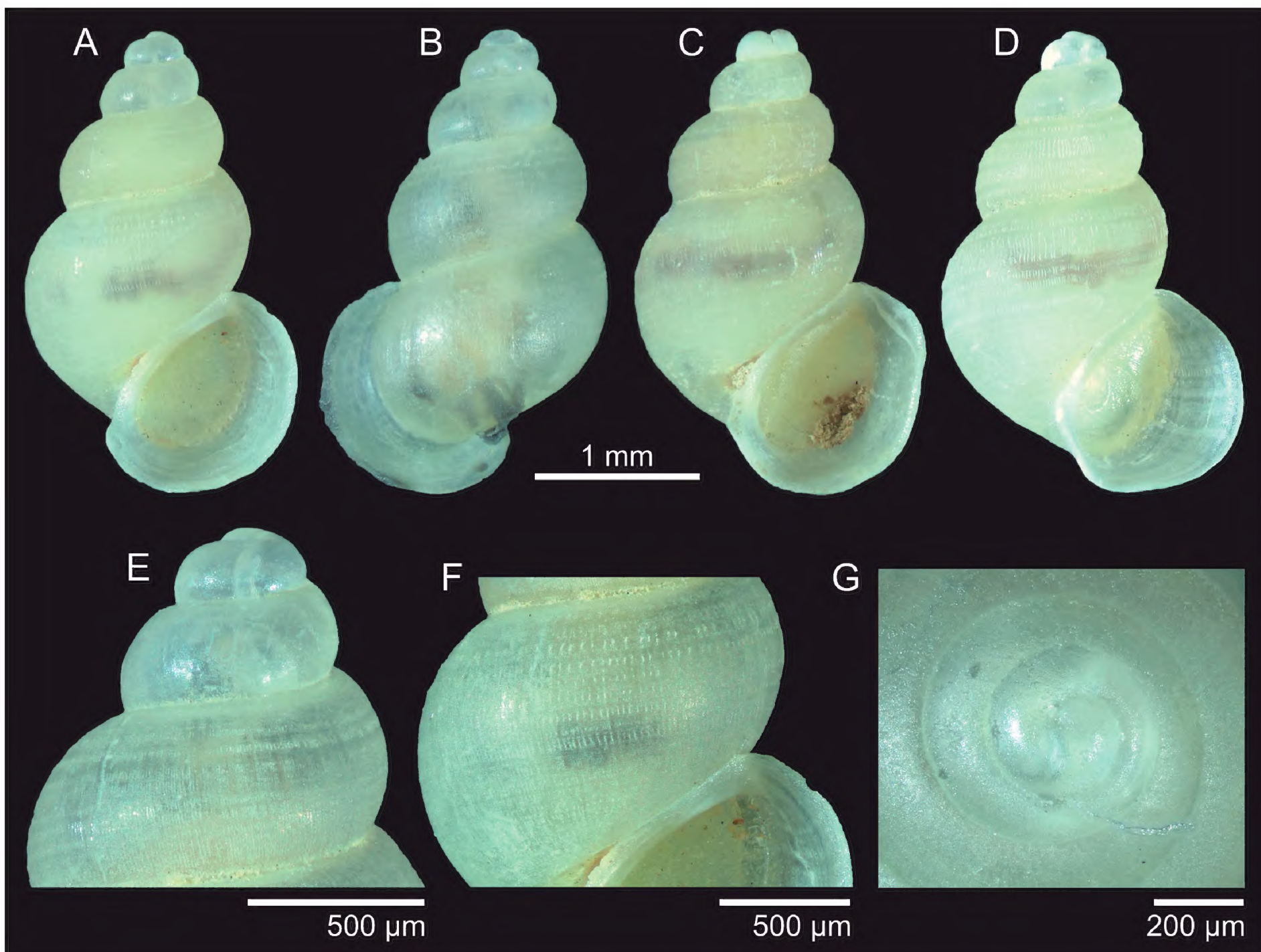


Figure 5. *Idiopyrgus meriadoci* sp. nov. **A, B.** Holotype LES 29798; **C.** Paratype #1 LES 29820; **D.** Paratype #2 LES 29820; **E.** Detail of the spire of the holotype in apertural view, showing the teleoconch sculpture in the early whorls; **F.** Detail of the body whorl of holotype in apertural view, showing the teleoconch sculpture; **G.** Detail of the protoconch of the holotype.

Etymology. The species is named in honour of Meriadoc “Merry” Brandybuck, from J.R.R. Tolkien’s “The Lord of the Rings.” Besides standing with Éowyn against the Witch-king in the Battle of the Pelennor Fields, Merry is also an example of the fight for nature conservation in Middle-earth, pushing the Ents into action and ultimately ending Saruman’s threat to Fangorn Forest.

Diagnosis. Shell minute, whitish, entirely translucent. Teleoconch sculptured by numerous spiral rows of minute vertical pustulae, arranged roughly equidistantly from one another, which do not form thorn-like periostracal hairs. Aperture oval, adapically and abapically angulated.

Description. Shell conical, $4\frac{1}{2}$ – $4\frac{3}{4}$ whorls, 2.8–2.9 mm high, 1.6–1.8 mm wide. Colour pale yellow to white; shell entirely translucent. Protoconch of $1\frac{1}{4}$ – $1\frac{1}{2}$ whorl (transition to teleoconch not easily observable), rounded, smooth (Fig. 5E,G). Suture deep. Whorls increasing uniformly in height and width. Teleoconch sculptured by numerous spiral rows of minute vertical pustules, arranged roughly equidistantly from one another (Fig. 5E,F); they do not develop thorn-like periostracal hairs as seen in some of its congeners (e.g., *I. eowynae* sp. nov.), though a few pustules on the body display what seem to be minute truncated hairs in two of

the more apically-positioned rows (Fig. 5F). Peristome complete, not covering penultimate whorl, expanded, wider in abapical region, narrower in columellar region, of same colour as body whorl. Aperture oval, adapically and abapically angulated, but with smooth, rounded contour. Umbilicus rimate, nearly closed. Operculum oval, paucispiral; corneous, thin, and translucent, of same colour as body whorl. Soft body completely white in colour.

Distribution. Known only from type locality (Fig. 1).

Remarks. *Idiopyrgus meriadoci* sp. nov. is easily distinguished from all its congeners by its unique teleoconch sculpture and much smaller shell; it is smaller than even *Idiopyrgus minor* Salvador, Silva & Bichuette, 2022, which was previously the species with the smallest body size in the genus. The whitish and entirely translucent shell, along with the smaller number of whorls, is likewise useful to easily distinguish it from *Idiopyrgus eowynae* sp. nov. (see above) and *I. umbraticola*. The latter two species also display a teleoconch sculpture that seems to be homologous to that of *I. meriadoci* sp. nov.; the latter, however, lacks the periostracal hairs observed in the former two species.

As for *I. eowynae* sp. nov. above, there is little variation in shell shape and proportions among the presently available specimens of *I. meriadoci* sp. nov. (Fig. 5A–D).

***Idiopyrgus umbraticola* (Simone & Salvador, 2021),
comb. nov.**

Spiripockia umbraticola Simone & Salvador, 2021: 3123, figs. 2, 3;
Salvador et al. 2022b: 2, figs. 8, 9.

Remarks. *Spiripockia umbraticola* is known only from the type locality, Gruna do Domingão cave (Carinhanha municipality, Serra do Ramalho region, Bahia state). When Simone and Salvador (2021) described that species, we still did not have the understanding of *Idiopyrgus* and of the family Tomichiidae in Brazil that we have today (Salvador et al. 2022a). Hence, those authors classified the new species in *Spiripockia*, heavily influenced by the presence of periostracal hairs on the teleoconch and by the fact that the animals were cavernicolous. Nevertheless, the overall shell shape of *Spiripockia umbraticola* was very different from *Spiripockia punctata* Simone, 2012, the type species of the genus and, at that point, the only species within it. The anatomical similarities between those two species are within expectations in Truncatelloidea, and some meaningful differences in the reproductive trait were observed by Simone and Salvador (2021).

Spiripockia umbraticola is morphologically very similar to *I. eowynae* sp. nov. (see above), which the present phylogenetic analysis has placed with full support within *Idiopyrgus* (Fig. 3). Thus, based on the new morphological and phylogenetic evidence, *Spiripockia umbraticola* is here reclassified as *Idiopyrgus umbraticola* (Simone & Salvador, 2021), comb. nov.

The genus *Spiripockia*, therefore, returns to being monotypic, including only *Spiripockia punctata*. However, considering the newly recognised Gondwanan family Tomichiidae in Brazil (Salvador et al. 2022a; Lawrie et al. 2023) and the new findings presented here, the family-level classification of *Spiripockia* (formerly Pomatiopsidae, presently Cochliopidae; Simone and Salvador 2021) and its status as a distinct genus needs to be reassessed. Previous attempts to obtain genetic sequences from this species failed (Simone and Salvador 2021), and new specimens must be obtained to that end. Furthermore, we lack a consensus of what the anatomical features of Brazilian Cochliopidae and Tomichiidae are, i.e., which are pleomorphic and which are apomorphic, and more importantly, which can be used as diagnostic features (a confusion already noted by Simone and Salvador 2021 in relation to Cochliopidae in general and other truncatelloid clades as well). That will only be achieved by a thorough anatomical study of both families in Brazil, alongside a solid phylogenetic backbone built on genetic data.

Discussion

Including the present findings, the genus *Idiopyrgus* now counts with ten species, half of which are restricted to or closely related to cave environments. Thus, *Idiopyrgus* is in an odd position of being a relict South American genus

of a Gondwanan family (Salvador et al. 2022a; Lawrie et al. 2023) but also having radiated into caves, where several different species arose. Undoubtedly, as the numerous caves in Brazil are explored (karsts are typical hotspots for subterranean biodiversity; Culver and Sket 2000; Sket 2016), more species are bound to come to light (Salvador et al. 2022b; Machado et al. 2023).

Troglomorphisms

In view of the presently available information, the new species described herein are endemic to a single cave (they have not been found in collections in other caves). *Idiopyrgus meriadoci* sp. nov. can be found in the twilight zone and dark (aphotic) zone of the cave; the typical morph of *I. eowynae* sp. nov. is found in the twilight and dark zones, while the dark morph is found closer to the cave entrance.

Considering the classification of subterranean organisms (Romero 2009; Christiansen 2012; Trajano and Carvalho 2017), we identify both *I. eowynae* sp. nov. and *I. meriadoci* sp. nov. as troglobitic species. Besides their endemism, there are other morphoanatomical features to support this, some of which are of particular importance for freshwater gastropods (Grego et al. 2020; Gladstone et al. 2018, 2021; Salvador et al. 2022b, 2024) as follows. *Idiopyrgus meriadoci* sp. nov. has a white translucent and fragile shell and unpigmented body; it also has a minute size, smaller than all other congeners. These are features typical of specialised cave fauna (Gladstone et al. 2018, 2021; Grego et al. 2020). Likewise, *I. eowynae* sp. nov. has an unpigmented body, and its typical morph has a translucent and fragile shell.

The dark morph of *I. eowynae* sp. nov. displays a sturdier black shell, which has also been observed in *I. adamanteus* and *I. cf. walkeri* (Salvador et al. 2022a). While the significance of this is yet unknown, similar colour polymorphism is also known in other troglobitic animals, particularly in gastropods of the genus *Potamolithus* and in fish (Bichuette and Trajano 2004, 2005, 2018). Considering that there is negligible genetic distance between the two morphs in their mitochondrial markers (Fig. 3), we can only hypothesise that would be true for the rest of the genome and that the morphological differences observed in the shell are likely due to phenotypic plasticity and/or have direct environmental causes.

Both *I. eowynae* sp. nov. and *I. meriadoci* sp. nov. have a unique teleoconch sculpture (i.e., rows of periostracal hairs), so far unknown in surface-dwelling freshwater snails in Brazil but known from other cave-dwelling snails in the country: *I. umbraticola* and *Spiripockia punctata* (Simone 2012; Simone and Salvador 2021; Salvador et al. 2022b). However, we are presently unable to say whether this is somehow related to life in a cave environment or not, but the fact that only cave snails display it might be an indication of that. Other members of *Idiopyrgus*, including cave- and surface-dwelling species, lack the sculpture (Salvador et al. 2022a), so future studies

could, for instance, show that the sculpture is a feature related to a clade within the genus (e.g., *I. eowynae* sp. nov. + *I. meriadoci* sp. nov. + *I. umbraticola*). Even so, according to our present phylogenetic analysis (Fig. 3), the cave-dwelling species *I. eowynae* sp. nov., *I. adamanteus*, and *I. minor* form a single clade. While this apparently could indicate that cave species are closely related and descend from a common ancestor, it should be noted that our coverage of the genus is still quite reduced, and future studies with a better sampling across more species and populations could paint a different picture.

The facts that *Idiopyrgus* is a relict genus and that it has radiated into caves, alongside the intraspecific (and potentially non-genetic) differences in shell morphology, make species in this genus good models for studies that go beyond phylogenetics and biogeography and into evolutionary ecology. Thus, we hope that our account will bring more attention to these intriguing animals.

Geographic distribution and natural history

The genus *Idiopyrgus* has a reasonable geographic range throughout Brazil, occurring mostly in the eastern part of the country (except for *I. brasiliensis* (Rey, 1959) in the west) and across five different hydrographic regions: São Francisco (where most diversity and records are), East Northeast Atlantic, Southeast Atlantic, Tocantins-Araguaia, and Paraguay (Salvador et al. 2022a: fig. 4). There is little data on the distribution of most species, but they seem to have restricted ranges, often known only from their type localities (Salvador et al. 2022a). The exception is *I. souleyetianus*, which is a widespread species, distributed across three states (Bahia, Minas Gerais, and Espírito Santo) and two hydrographic regions (São Francisco and Southeast Atlantic) (Salvador et al. 2022a).

Three of the previously known species (*I. adamanteus*, *I. minor*, and *I. umbraticola*) are so far known only from caves, while *I. souleyetianus* and potentially *I. walkeri* have wider distributions and have also been recorded from caves (Salvador et al. 2022a). Notably, *I. umbraticola* and *I. minor* occur in the Serra do Ramalho region near the municipality of Carinhanha, in which both new species described herein (as well as *Spiripockia punctata*) can be found (Fig. 2). The region of Central, to the north of Carinhanha and where *I. adamanteus* can be found, is dominated by the Caatinga biome with a dry vegetation type, and the caves have been hypothesised as refuges for these animals (Salvador et al. 2022a).

Considering the difficulty of accessing the caves and conducting detailed observations of live animals, so far only snapshots of their biology are known. We collated here all the information currently available from the literature and from field observations by MEB.

Idiopyrgus umbraticola occurs only in the aphotic zone of Gruna do Domingão cave, attached to hard surfaces such as rocks and logs in the water (Simone and Salvador 2021). This species co-occurs with (and uses the same

microhabitat as) *Xangoniscus aganju* Campos-Filho, Araujo & Taiti, 2014 (Campos-Filho et al. 2014), an amphibious troglobitic isopod.

Both *I. eowynae* sp. nov. and *I. meriadoci* sp. nov. were found side by side in a small stream inside the Gruna do Pedro Cassiano cave, attached to the small roots of plants (e.g., “gameleiras”) that penetrate the caves from the surface in search of water; the deep of the small lentic drainage is formed by silt and has a depth of 10 to 15 cm. Values of pH and temperature measured on site varied, respectively, from 7.5 to 8.2 and from 21.3 to 22.4 °C. Both snail species occur in the cave’s twilight zone and dark (aphotic) zones; however, the dark morph of *I. eowynae* sp. nov. lives close to the cave entrance. It is interesting that both species also co-occur with a species of the isopod genus *Xangoniscus*, probably *X. aganju* (Fig. 1G), and the cave catfish species *Trichomycterus*, probably *T. rubbioli* (Bichuette & Rizzato, 2012) (Fig. 1H).

Idiopyrgus minor lives in small pools resulting from infiltration water in the aphotic zone of two close-by caves (Gruna da Pingueira cave and Gruna do Engrunado cave) in Feira da Mata municipality and is apparently restricted to this type of habitat (Salvador et al. 2022a). *Idiopyrgus adamanteus* occurs in four close-by caves in Central municipality (Toca Bonita cave, Toca da Mulher cave, Toca de Candeias cave, and Toca da Machadinha cave) (Salvador et al. 2022a); individuals of this species were always found in phreatic waters, in twilight and aphotic zones of the caves, attached to rocks and submerged logs.

Spiripockia punctata lives attached to rocks inside a small stream in the aphotic zone in the Lapa dos Peixes cave (Simone 2012), also co-occurring with the isopod *X. aganju* and a species of cave catfish, *Trichomycterus rubbioli*. Both the isopod and catfish are troglobites (Bichuette and Rizzato 2012; Campos-Filho et al. 2014).

The repeated co-occurrence of *Idiopyrgus* and the isopod *Xangoniscus aganju* is interesting. The genus *Xangoniscus* is composed only by troglobitic species with high endemism (Campos-Filho et al. 2014; Cardoso et al. 2020), and the somewhat more widespread *X. aganju* is suspected of being a cryptic species complex (MEB, pers. obs.). This partially parallels the story of *Idiopyrgus* in the region (Salvador et al. 2022a). Also, the observation that *Idiopyrgus* spp. and *Xangoniscus* spp. share the same microhabitats begs the investigation of other potential relationships between these species, such as modes of dispersal and cave colonisation.

Conservation concerns

Considering the co-occurrence of two species in the same cave and sharing the same microhabitat, the threats observed regionally and locally such as water use, deforestation in the cave surroundings, and the progressively decreasing recharge of water to the cave likely due to climate changes (Fig. 1A; Gallão and Bichuette 2018), we can consider both species to be in a vulnerable situation.

Some impacts are specific, such as the extraction of water (Fig. 1D) for use in villages and plantations, but they are nonetheless worrying (Gallão and Bichuette 2018). The fact that both species are endemic to the same locality already makes them fragile to any environmental disturbance. In addition, the cave is about 2 km long, considering the mapped passageways (Fig. 1C, cave gallery of Gruna do Pedro Cassiano; Grupo Bambuí de Pesquisas Espeleológicas, pers. comm.), reaching a potential extension area, for both species, of 2,000 m². However, from a small-scale perspective, despite the high abundance observed, with population densities reaching an average of 30 individuals/m² (estimated *in loco* during fieldwork), both species use specific microhabitats with a high concentration of roots (Fig. 1E, F), which implies a small area of occupation not exceeding 20 m² and which further increases their risk of extinction.

Conclusion

Based on our results, we described two new troglobitic species of *Idiopyrgus* and transferred a third species from *Spiripockia* to it. This reinforces the presence of the family Tomichiidae in Brazil, representing a relict genus that largely radiated into cave environments. Thus, building upon the revision of Salvador et al. (2022a), the genus *Idiopyrgus* now contains the following ten species:

- *Idiopyrgus souleyetianus* Pilsbry, 1911 [type species];
- *Idiopyrgus adamanteus* Salvador, Silva & Bichuette, 2022;
- *Idiopyrgus brasiliensis* (Rey, 1959);
- *Idiopyrgus eowynae* sp. nov.;
- *Idiopyrgus meriadoci* sp. nov.;
- *Idiopyrgus minor* Salvador, Silva & Bichuette, 2022;
- *Idiopyrgus pilsbryi* Baker, 1914;
- *Idiopyrgus rudolphi* (Haas, 1938);
- *Idiopyrgus umbraticola* (Simone & Salvador, 2021), comb. nov.;
- *Idiopyrgus walkeri* Pilsbry, 1924 [taxon inquirendum].

Acknowledgements

We thank Paulo Ricardo S. Coelho (UFMG, Brazil) for providing the sample of *Idiopyrgus souleyetianus*; the Department of Arctic and Marine Biology (UiT, Norway) for the support with the genetic analyses; CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support and productivity fellowship to MEB (#313332/2023-1); SISBIO (Sistema de Autorização e Informação em Biodiversidade) for collection permit to MEB (#28992); ICMBio/CECAV (Instituto Chico Mendes para Conservação da Biodiversidade/Centro Nacional de Pesquisa e Conservação de Cavernas) for financial support from environmental compensation

funds through the TCCE Vale and ICMBio agreement to the projects “Estudos para definição de áreas prioritárias para a Conservação de Proteção Espeleológico na Serra do Ramalho – BA”, executed by the GBPE (Grupo Bambuí de Pesquisas Espeleológicas) and “Teste de metodologias propostas em legislação ambiental relacionadas à fauna subterrânea e proposição de novas áreas prioritárias para conservação de cavernas” under execution by MEB; and to the three reviewers for their comments and suggestions (even though Reviewer 3 was a LOTR “hater”). We especially thank Jonas E. Gallão for all support in the field trips and collections in Serra do Ramalho region, and to Jessica S. Gallo for confection of the map of Fig. 2. Open access funded by Helsinki University Library.

References

- Attwood SW, Ambu S, Meng XH, Upatham ES, Xu FS, Southgate VR (2003) The phylogenetics of triculine snails (Rissooidea, Pomatiopsidae) from south-east Asia and southern China: historical biogeography and the transmission of human schistosomiasis. *Journal of Molluscan Studies* 69(3): 263–271. <https://doi.org/10.1093/mollus/69.3.263>
- Bedeck HE, Zimmermann NE, McVicar TR, Vergopolan N, Berg A, Wood EF (2018) Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data* 5: e180214. <https://doi.org/10.1038/sdata.2018.214>
- Bichuette ME, Rizzato PP (2012) A new species of cave catfish from Brazil, *Trichomycterus rubbioli* sp.n., from Serra do Ramalho karstic area, São Francisco River basin, Bahia State (Siluriformes, Trichomycteridae). *Zootaxa* 3480: 48–66. <https://doi.org/10.11646/zootaxa.3480.1.2>
- Bichuette ME, Trajano E (2004) Three new subterranean species of *Ituglanis* from Central Brazil (Siluriformes, Trichomycteridae). *Ichthyological Exploration of Freshwaters* 15(3): 243–256.
- Bichuette ME, Trajano E (2005) A new cave species of *Rhamdia* Bleeker, 1858 (Siluriformes, Heptapteridae) from Serra do Ramalho, northeastern Brazil, with notes on ecology and behavior. *Neotropical Ichthyology* 3(4): 587–595. <https://doi.org/10.1590/S1679-62252005000400016>
- Bichuette ME, Trajano E (2018) Diversity of *Potamolithus* (Littorinimorpha, Truncatelloidea) in a high-diversity spot for troglobites in southeastern Brazil: role of habitat fragmentation in the origin of subterranean fauna, and conservation status. *Subterranean Biology* 25: 61–88. <https://doi.org/10.3897/subtbiol.25.23778>
- Campos-Filho IS, Araujo PB, Bichuette ME, Trajano E, Taiti S (2014) Terrestrial isopods (Crustacea, Isopoda, Oniscidea) from Brazilian caves. *Zoological Journal of the Linnean Society* 172(2): 360–425. <https://doi.org/10.1111/zoj.12172>
- Cardoso GM, Bastos-Pereira R, Souza LA, Ferreira RL (2020) New troglobitic species of *Xangoniscus* (Isopoda, Styroniscidae) from Brazil, with notes on their habitats and threats. *Zootaxa* 4819: 84–108. <https://doi.org/10.11646/zootaxa.4819.1.4>
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17(4): 540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>

- Christiansen K (2012) Morphological adaptations. In: Culver DC, White WB (Eds) Encyclopedia of Caves. Elsevier Academic Press, Amsterdam, 2nd ed., 517–528. <https://doi.org/10.1016/B978-0-12-383832-2.00075-X>
- Criscione F, Ponder WF (2013) A phylogenetic analysis of rissooidean and cingulopsoidean families (Gastropoda, Caenogastropoda). *Molecular Phylogenetics and Evolution* 66(3): 1075–1082. <https://doi.org/10.1016/j.ympev.2012.11.026>
- Culver DC, Sket B (2000) Hotspots of subterranean biodiversity in caves and wells. *Journal of Cave and Karst Studies* 62: 11–17.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Gallão JE, Bichuette ME (2018) Brazilian obligatory subterranean fauna and threats to the hypogean environment. *Zookeys* 746: 1–23. <https://doi.org/10.3897/zookeys.746.15140>
- Gladstone NS, Carter ET, McKinney ML, Niemiller ML (2018) Status and distribution of the cave-obligate land snails in the Appalachians and interior Low Plateau of the eastern United States. *American Malacological Bulletin* 36: 62–78. <https://doi.org/10.4003/006.036.0107>
- Gladstone NS, Niemiller ML, Hutchins B, Schwartz B, Czaja A, Slay ME, Whelan NV (2021) Subterranean freshwater gastropod biodiversity and conservation in the United States and Mexico. *Conservation Biology* 36(1): e13722. <https://doi.org/10.1111/cobi.13722>
- Grego J, Mumladze L, Falniowski A, Osikowski A, Rysiewska A, Palatov DM, Hofman S (2020) Revealing the stygobiotic and crenobiotic molluscan biodiversity hotspot in Caucasus: Part I. The phylogeny of stygobiotic Sadlerianinae Szarowska, 2006 (Mollusca, Gastropoda, Hydrobiidae) from Georgia with descriptions of five new genera and twenty-one new species. *ZooKeys* 955: 1–77. <https://doi.org/10.3897/zookeys.955.51983>
- Guan F, Niu AO, Attwood SW, Li YL, Zhang B, Zhu YH (2008) Molecular phylogenetics of triculine snails (Gastropoda, Pomatiopsidae) from southern China. *Molecular Phylogenetics and Evolution* 48(2): 702–707. <https://doi.org/10.1016/j.ympev.2008.04.021>
- Hershler R, Liu H-P, Mulvey M (1999) Phylogenetic relationships within the aquatic snail genus *Tryonia*: implications for biogeography of the North American Southwest. *Molecular Phylogenetics and Evolution* 13: 377–391. <https://doi.org/10.1006/mpev.1999.0659>
- Kameda Y, Kato M (2011) Terrestrial invasion of pomatiopsid gastropods in the heavy-snow region of the Japanese Archipelago. *BMC Evolutionary Biology* 11(1): e118. <https://doi.org/10.1186/1471-2148-11-118>
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Kroll O, Hershler R, Albrecht C, Terrazas EM, Apaza R, Fuentealba C, Wolff C, Wilke T (2012) The endemic gastropod fauna of Lake Titicaca: correlation between molecular evolution and hydrographic history. *Ecology and Evolution* 2: 1517–1530. <https://doi.org/10.1002/ece3.280>
- Lawrie AD, Chaplin J, Kirkendale L, Whisson C, Pinder A, Mlambo MC (2023) Phylogenetic assessment of the halophilic Australian gastropod *Coxiella* and South African *Tomichia* resolves taxonomic uncertainties, uncovers new species and supports a Gondwanan link. *Molecular Phylogenetics and Evolution* 184: 107810. <https://doi.org/10.1016/j.ympev.2023.107810>
- Machado FM, Miranda MS, Salvador RB, Pimenta AD, Côrtes MO, Gomes JAJ, Miyahira IC, Agudo-Padrón I, Oliveira CDC, Caetano CHS, Coelho PRS, D’ávila S, Arruda EP, Almeida SM, Gomes SR, Alvim J, Filho HG, Ferreira-Júnior AL, Marques RC, Martins I, Souza LS, Arruda JO, Cavallari DC, Santos SB, Pedro NC, Salles ACA, Dornellas APS, Lima TC, Amaral VS, Silva FS, Passos FD, Thiengo SS, Leite TS, Simone LRL (2023) How many species of Mollusca are there in Brazil? A collective taxonomic effort to reveal this still unknown diversity. *Zoologia* 40: e23026. <https://doi.org/10.1590/s1984-4689.v40.e23026>
- Miller MA, Schwartz T, Pickett BE, He S, Klem EB, Scheuermann RH, Passarotti M, Kaufman S, O’Leary MA (2015) A RESTful API for access to phylogenetic tools via the CIPRES Science Gateway. *Evolutionary Bioinformatics Online* 11: 43–48. <https://doi.org/10.4137/EBO.S21501>
- Ponder WF, Wilke T, Zhang W-H, Golding RE, Fukuda H, Mason RAB (2008) *Edgbastonia alanwillsi* n. gen & n. sp. (Tateinae, Hydrobiidae s.l., Rissooidea, Caenogastropoda); a snail from an artesian spring group in western Queensland, Australia, convergent with some Asian Amnicolidae. *Molluscan Research* 28: 89–106. <https://doi.org/10.11646/mr.28.2.1>
- Romero A (2009) Cave Biology: Life in Darkness. Cambridge University Press, Cambridge, 306 pp. <https://doi.org/10.1017/CBO9780511596841>
- Ronquist F, van der Mark P, Huelsenbeck JP (2009) Bayesian phylogenetic analysis using MrBayes. In: Lemey P, Salemi M, Vandamme A-M (Eds) The Phylogenetic Handbook: a Practical Approach to Phylogenetic Analysis and Hypothesis Testing. Cambridge University Press, Cambridge, 210–266. <https://doi.org/10.1017/CBO9780511819049.009>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Salvador RB, Silva FS, Bichuette ME (2022a) Phylogenetic position of the relict South American genus *Idiopyrgus* Pilsbry, 1911 (Gastropoda, Truncatelloidea), with the description of two new cave species. *Zoosystematics and Evolution* 98(2): 365–375. <https://doi.org/10.3897/zse.98.90797>
- Salvador RB, Silva FS, Cavallari DC, Cunha CM, Bichuette ME (2022b) Cave-dwelling gastropods (Mollusca, Gastropoda) of Brazil: state of the art and conservation. *Zoologia (Curitiba)* 39: e21033. <https://doi.org/10.1590/s1984-4689.v39.e21033>
- Salvador RB, Silva FS, Cavallari DC, Cunha CM, Bichuette ME (2024) Cave-dwelling gastropods of Brazil: a reply to Ferreira et al. (2023). *Zoologia* 41: e23099. <https://doi.org/10.1590/s1984-4689.v41.e23099>
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and compilation of conserved Polymerase Chain Reaction primers. *Annals of the Entomological Society of America* 87: 651–701. <https://doi.org/10.1093/aesa/87.6.651>

- Simone LRL (2006) Land and freshwater Molluscs of Brazil. Fapesp, São Paulo, 390 pp.
- Simone LRL (2012) A new genus and species of cavernicolous Pomatiopsidae (Mollusca, Caenogastropoda) in Bahia, Brazil. *Papeis Avulsos de Zoologia* 52(40): 515–524. <https://doi.org/10.1590/S0031-10492012022000001>
- Simone LRL, Salvador RB (2021) A new species of *Spiripockia* from eastern Brazil and reassignment to Cochliopidae (Gastropoda, Truncatelloidea). *Journal of Natural History* 54(47–48): 3121–3130. <https://doi.org/10.1080/00222933.2021.1890850>
- Sket B (2016) Subterranean (hypogean) habitats in karst and their fauna. In: Finlayson C, Milton G, Prentice R, Davidson N (Eds) *The Wetland Book*. Springer, Dordrecht, 1–14. https://doi.org/10.1007/978-94-007-6173-5_241-1
- Takano T, Kano Y (2014) Molecular phylogenetic investigations of the relationships of the echinoderm-parasite family Eulimidae within Hypsogastropoda (Mollusca). *Molecular Phylogenetics and Evolution* 79: 258–269. <https://doi.org/10.1016/j.ympev.2014.06.021>
- Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56(4): 564–577. <https://doi.org/10.1080/10635150701472164>
- Trajano E, Carvalho MR (2017) Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. *Subterranean Biology* 22: 1–26. <https://doi.org/10.3897/subtbiol.22.9759>
- Wade CM, Mordan PB (2000) Evolution within the gastropod molluscs: using the ribosomal RNA gene cluster as an indicator of phylogenetic relationships. *Journal of Molluscan Studies* 66: 565–570. <https://doi.org/10.1093/mollus/66.4.565>
- Wade CM, Mordan PB, Naggs F (2006) Evolutionary relationships among the pulmonate land snails and slugs (Pulmonata, Stylommatophora). *Biological Journal of the Linnean Society* 87: 593–610. <https://doi.org/10.1111/j.1095-8312.2006.00596.x>
- Wilke T, Davis GM, Gong X, Liu H-X (2000) *Erhaia* (Gastropoda, Risssooidea): phylogenetic relationships and the question of *Paragonimus* coevolution in Asia. *American Journal of Tropical Medicine and Hygiene* 62: 453–459. <https://doi.org/10.4269/ajtmh.2000.62.453>
- Wilke T, Davis GM, Falniowski A, Giusti F, Bodon M, Szarowska M (2001) Molecular systematics of Hydrobiidae (Mollusca, Gastropoda, Risssooidea): testing monophyly and phylogenetic relationships. *Proceedings. Academy of Natural Sciences of Philadelphia* 151(1): 1–21. [https://doi.org/10.1635/0097-3157\(2001\)151\[0001:MSOHMG\]2.0.CO;2](https://doi.org/10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2)
- Wilke T, Haase M, Hershler R, Liu H-P, Misof B, Ponder W (2013) Pushing short DNA fragments to the limit: phylogenetic relationships of ‘hydrobioid’ gastropods (Caenogastropoda, Risssooidea). *Molecular Phylogenetics and Evolution* 66(3): 715–736. <https://doi.org/10.1016/j.ympev.2012.10.025>